REMARKS

Amendments

In the specification, the paragraph at page 4, lines 23-24 has been amended to be consistent with the formal drawing for Figure 14, as suggested by the Examiner.

Claims 5, 6, 8, 9, 11, 24, and 25 remain in this application. Claims 1-4, 7, and 15 have been previously cancelled. Claims 10, 12-14, 16-23, and 26 have been withdrawn as the result of an earlier restriction requirement. Claims 5, 6, and 24 are amended. No new matter is added by the amendments, which correct grammatical or antecedent basis errors.

In view of the Examiner's earlier restriction requirement, which has been made final, applicants retain the right to present withdrawn claims 10, 12-14, 16-23, and 26 in a divisional application or to re-present certain of them if the elected species is found patentable.

Specification Objections

The disclosure is objected to because Figure 14 should be described as Figures 14A and B. The specification is amended to reflect this change.

The amendment filed on November 30, 2001 in Paper No. 9 is objected to under 35 U.S.C. §132 because, according to the Examiner, the three new paragraphs requested to be inserted at page 20, line 11 of the specification introduce new matter into the disclosure. Applicants respectfully traverse this rejection.

Figures 19-21 were not submitted with the above application as filed, and applicants received a Notice of Omitted Items mailed May 30, 2001 indicating that Figures 19 and 20 (but not 21) were missing. Under MPEP section 601.01(d), applicants willing to accept the application as deposited in the USPTO need not respond to the Notice of Omitted Items. If applicants elect not so to respond, applicants are required to amend the specification to cancel all references to omitted drawings in a preliminary amendment. The present applicants complied with this requirement by amending the specification in the preliminary amendment filed November 30, 2001.

These three introduced paragraphs are simply derived from the legends for Figures 19-21 that appeared in the original filing beginning at page 4, line 33 and ending at page 5, line 22 of the

specification. Whether or not those **figures** were present when the application was filed, the corresponding **figure legends** unambiguously constitute part of the original disclosure. Because these paragraphs were amended to delete reference to the figures, they do not belong in the Brief Description of the Drawings section, but somewhere else. Applicants chose to move these three paragraphs to the Examples section where the figures are referred to in the original filing. If the Examiner believes these paragraphs belong in another place of the application, applicants welcome recommendations for a better location.

The experimental procedures in these three inserted paragraphs to which the Examiner refers on the bottom of page 3 of the Office Action are the same procedures described in the original figure legends. The only differences between these two are that the Figure numbers and any reference to or information dependent on a figure have been removed, as required by the MPEP. Therefore, no new matter is introduced into the disclosure by these paragraphs, and applicants respectfully request that the objections to the disclosure/amendment be reconsidered and withdrawn.

Claim Objections

Claims 5 and 24 are objected to because of the language at line 3, "initiation region variant operably linked to nucleic acid." The Examiner urges that proper grammar dictates that the word "a" be inserted before "nucleic acid." This has now been effected in both claims, without changing the scope of the claims. Hence, applicants respectfully request reconsideration and withdrawal of the objection to the claims.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 5, 6, and 24 (and dependent claims 8, 9, 11, and 25) are rejected under 35 U.S.C. §112, second paragraph, for various reasons set forth below:

1. Claims 5 and 24 are allegedly incomplete for omitting essential elements. According to the Office, while the preamble recites a method of secreting a heterologous polypeptide at lines 1-2, the method steps conclude with expressing a heterologous polypeptide at line 4, thereby omitting a subsequent secretion st p resulting from the

method. For clarification, but not changing the scope of the claims, applicants have now inserted a secretion step into the methods of claims 5 and 24.

2. Claim 5 recites "the wild-type" in line 8, which has insufficient basis in the claim, according to the Examiner. In response, claim 5 and claim 24 (which contains the same language and is independent) have been amended to recite "a" wild-type. This amendment does not change the scope of the claims.

3. Claim 6 is said to have an internal inconsistency regarding linkage. Hence, claim 6 is amended to remove the conditional linkage language, since the nucleic acid is already linked. This amendment does not alter the claim scope, but rather clarifies the meaning thereof.

In view of the claim amendments, applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

Rejection Under 35 U.S.C. §102(b) (Klein *et al.*)

Claims 5, 8, 9, 11, 24, and 25 are rejected under 35 U.S.C. §102(b) as being anticipated by Klein *et al.*, Protein Engineering, Vol. 5: 511-517 (1992), hereafter "Klein."

The Examiner states that Klein teaches a secretion signal polypeptide (LamB) fused to a heterologous polypeptide, bovine somatotropin (bST). The secretion signal polypeptide is a variant of the wild-type secretion signal polypeptide, and the fused polypeptide is expressed and secreted.

Applicants respectfully traverse this rejection. Anticipation requires that all of the elements and limitations of the claims be found within a single prior art reference. There must be no difference between the claimed invention and the reference disclosure as viewed by one of ordinary skill in the art. *Scripps Clinic & Research Fdn. v. Genentech*, 927 F.2d 1565, 1576 (Fed. Cir. 1991) (emphasis added). Absence from a cited reference of any element of a claim negates anticipation of that claim by that reference. *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 224 USPQ 409 (Fed. Cir. 1984).

Applicants note that claims 5 and 24, upon which all other rejected claims ultimately depend, both specify that the translational

strength of the translational initiation region of the variant secretion signal polypeptide is less than the translational strength of a wild-type translational initiation region. To evaluate the levels of secretion of bST, Klein made various constructs containing random mutations in the C-terminal half of the lamB signal peptide that is not part of the translational initiation region. Since the translational initiation region and its strength remain constant in these mutation experiments, none of the random mutations Klein made conferred a lower translational strength on the translational initiation region than that of the comparable wild-type region.

Hence, nowhere does Klein disclose the particular element of the present claims regarding lower translational strength as compared to wild-type. Because Klein does not disclose each and every element of the claimed invention, applicants respectfully request reconsideration and withdrawal of the rejection of claims 5, 8, 9, 11, 24, and 25 under 35 U.S.C. §102(b) over Klein.

Rejection Under 35 U.S.C. §102(b) (Benson *et al.*)

Claims 5, 8, 9, 11, 24, and 25 are rejected under 35 U.S.C. § 102(b) as being anticipated by Benson *et al.*, J. Bacteriol., Vol. 169: 4686-4691 (1987), hereafter "Benson."

Benson is cited for its teaching, in the abstract and the introduction, of a secretion signal polypeptide (LamB) fused to a heterologous polypeptide (LamB-LacZ), wherein the LamB signal polypeptide is a variant of the wild-type signal polypeptide and the fused polypeptide is expressed and secreted.

Applicants respectfully traverse this rejection. As noted above for Klein, claims 5 and 24, upon which all other rejected claims ultimately depend, both specify that the translational strength of the translational initiation region of the variant secretion signal polypeptide is less than that of a wild-type translational initiation region. Nowhere does Benson teach this particular element of the claims, and its variants do not have that feature.

The wild-type lamB signal sequence (designated MC4100) and all four mutants that Benson discusses, i.e., MC4100(701-708), MH8028, MH8021, and MH8023, have the same translational strength of the translational initiation region. The lamB701-708 mutant signal

sequence that is within the genotype of all four mutants contains two mutations that individually have translation initiation defects (decreased translation initiation), but the two mutations cancel each other out (see the first paragraph of the Results on page 4687, left col., bottom, to right col., top, and the second paragraph of the Discussion on page 4689, left col., bottom, to right col., top). Therefore, the translational strength of the genotype *lamB701-708* (mutant MC4100(701-708)) is the same as that of wild-type lamB. While Benson claims there is a connection between export and translation (see, e.g., the abstract), in fact, the connection is between export and translation elongation, not translation initiation, as is clear from the context and the statement on page 4689, right col., top) that the lamB701-708 mutation is a defect in translation elongation.

The second Benson mutant (MH8028) changes the amino acid sequence back to wild type (Ser to Arg), but retains the two mutations (lamB701-708) that cancel out translation initiation defects - and thus this mutant has the wild-type phenotype with no change in translational strength (see its genotype in Table 4 on page 4689). Similarly, the last two mutants of Benson (MH8021 and MH8023) retain the *lamB701-708* genotype and hence the translational initiation region and translational strength of the wild-type counterpart. The additional mutations in their respective genotypes occur in the hydrophobic core and downstream of the translational initiation region (see Table 4, page 4689 for the genotypes and page 4689, right col., first sentence of first full paragraph regarding the hydrophobic core), and therefore do not affect the translational strength.

In conclusion, all of the lamB signal sequence constructs discussed by Benson have the same translational strength, i.e., that of wild-type. Since anticipation requires that all the elements of the claimed invention must be disclosed in the single reference, as noted above, Benson does not destroy the novelty of the rejected claims. Hence, applicants respectfully request reconsideration and withdrawal of the rejection of claims 5, 8, 9, 11, 24, and 25 under 35 U.S.C. §102(b) over Benson.

Rejection Under 35 U.S.C. §103(a) (Klein in view of Goldstein et al.)

Claims 5, 6, 8, 9, 11, 24 and 25 are rejected under 35 U.S.C. §103(a) as being unpatentable over Klein in view of Goldstein et al., J. Bacteriol., Vol. 172: 1225-1231 (1990), hereafter "Goldstein."

While admittedly Klein does not teach that the amount of secreted polypeptide produced by the variant translational initiation region is greater than the amount of polypeptide secreted by the wild-type translational initiation region, Klein is cited for teaching the use of LamB and OmpA secretion signal polypeptides in side-by-side comparisons, demonstrating the obviousness of using LamB or OmpA to direct secretion of a heterologous polypeptide.

Goldstein is cited for its disclosure of a variant OmpA secretion signal polypeptide that induces greater amounts of secreted heterologous polypeptide than the wild-type counterpart, and for its teaching that the LamB secretion signal sequence has a comparable mechanism of action and similarity of structure to the OmpA secretion signal sequence, providing a rationale for the increased production of the heterologous polypeptide using the OmpA secretion signal sequence.

According to the Examiner, it would have been obvious to substitute the OmpA secretion signal polypeptide of Goldstein for the LamB secretion signal polypeptide of Klein for the expected benefit of increasing the production of the secreted heterologous polypeptide.

Applicants respectfully traverse this rejection. A finding of obviousness under 35 U.S.C. §103 requires a determination of the scope and content of the prior art, the differences between the invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. Deere*, 383 U.S. 1 (1966). Other considerations, such as unexpected results, failure of others, etc., that are indicia of non-obviousness must be taken into account, if present. *Fromson v. Advance Offset Plate, Inc.*, 755 F.2d 894, 904 (Fed. Cir. 1988). Once the scope and content of the prior art is determined, the relevant inquiry is whether the prior art as a whole suggests the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful. *In re Vaeck*, 20 USPQ 2d 1438 (Fed. Cir. 1991).

When references are combined to support a rejection, there must be some teaching in the references themselves that suggests the combination. *In re Sernaker*, 217 USPQ 1 (Fed. Cir. 1983).

The deficiencies of Klein are discussed above in the context of the novelty rejection. In particular, Klein does not disclose or suggest variants with a key feature of the two rejected independent claims, namely, that the translational strength of the variant translational initiation region is less than the translational strength of a wild-type translational initiation region. This is an unexpected feature of the claimed methods, as it would have been surprising at the relevant time period that one could secrete a polypeptide using a signal sequence variant with lower translational strength.

Goldstein has no relevance, even tangentially, to the present claims. Goldstein discloses amino acid mutations in the hydrophobic region of the OmpA signal sequence, to explore the role of this region in the rate of precursor processing, i.e., the rate of cleavage of the signal peptide from the mature protein. Hydrophobicity of the hydrophobic core of a signal sequence is correlated by Goldstein to processing of the signal peptide (generally agreed to be translocation rate), with overall hydrophobicity and structural conformational requirements stated to be important in signal function. Whereas Goldstein is changing the hydrophobic core of a signal sequence to explore precursor processing, the present applicants are changing the translational initiation region (and also translational strength) of a signal sequence to affect secretion. Goldstein does not even mention translational initiation in his paper, let alone suggest how it could be varied to accomplish the purpose set forth in the present claims.

In addition, Goldstein does not show any secretion data. Rather, media samples containing cells were precipitated with trichloroacetic acid and immunoprecipitated; that is, no supernatant fractions were made. In contrast, applicants' claims relate to secretion.

Hence, Goldstein is completely non-analogous art that the skilled molecular biologist would not have considered as relevant in any way to the t aching of Klein at the appropriate filing date. No rationale or incentive would have existed to substitute one signal sequence for another in these references in view of their divergent disclosures.

With respect to claim 6, the cited combination of art contains no suggestion and provides no motivation, or a reasonable expectation of success, that the claimed use of a variant secretion signal with lower translational strength than that of the native signal would still produce greater amounts of secreted heterologous polypeptide. The data reported by Klein on the 12 amino acid variants tested shows that none of the variants resulted in an increase in secretion: "the resulting bST levels can be divided into three broad categories: essentially parental levels, reduced soluble bST release (~25-50% of parental values) and no protein release" (see page 515 column 1, third paragraph); see also Figure 8 on page 516.

Klein also states that:

> [o]n re-assay, no clone showed significantly more bST release than the parental expression plasmid. Approximately 40% of the clones which contained amino acid substitutions showed parental levels of bST release, the remaining 60% being split between clones exhibiting reduced release and those which showed no bST in the periplasmic fraction (see page 515, column 1, third paragraph).

Klein concludes that "[t]he fact that no clones with increased secretion were identified may indicate that the signal peptide is not limiting for the secretion of the LamB-bST fusion." (see page 516, first paragraph).

As non-analogous art, Goldstein does not even refer to translation initiation as set forth above. Further, Goldstein does not make any measurements concerning the amount of mature protein either made or secreted; rather, Goldstein is only interested in how fast signal cleavage occurs. That is, Goldstein does not make any end point determinations of whether more protein is made or secreted with one mutant versus another. In fact, if anything, the gels shown in Figure 2 would suggest that the wild-type signal sequence results in the most mature protein produced, as the wild-type bands of mature protein appear the greatest in most or all of the gels.

Hence, since the combined disclosures of Klein and Goldstein teach away from increased secretion using variant signal sequences, they would not have provided a reasonable expectation of success of the method of claim 6.

In summary, the skilled artisan would not have been motivated to combine the teachings of Klein with those of Goldstein, since they cannot be meaningfully combined to arrive at the claimed methods with their unexpected feature. There would have been no reasonable expectation of success, given that the translational strength of the variants is lower than that of the wild-type signal sequence polypeptide in the presently claimed secretion methods, represented by claims 5 and 24. Further, for the reasons noted above, claim 6 would not have been obvious over the combination of Klein and Goldstein.

Since neither the primary reference nor the secondary reference, alone or in combination, provides the motivation for the claimed methods and contain no teaching or suggestion directed thereto, they do not meet the test for obviousness set forth above. Hence, applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) over Klein in view of Goldstein.

Information Disclosure Statement

An Information Disclosure Statement is being sent separately to the Office citing the three US patents (US 5,747,662; 5,840,523; and 6,242,177) that are related to this application, including the parent.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at the number indicated below.

This amendment is submitted with a transmittal letter and petition and fee for a one-month extension of time. Should this amendment be separated therefrom, and/or if fees are required, applicants petition the Commissioner to authorize charging Deposit Account 07-0630 for any fees required or credits due and any extensions of time necessary to maintain the pendency of this application.

Applicants believe the claims are in allowable form and respectfully solicit a Notice to that effect.

Respectfully submitted,
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Date: Nov 6, 2003

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